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13. ABSTRACT *(Maximum 200 Words)*

Androgen ablation therapy and anti-androgenic drugs represent the principal treatments for metastatic prostate cancer. However, nearly all tumors eventually relapse as hormone-refractory disease. A need therefore exists for better understanding of the mechanisms that allow prostate cancer cells to grow in an androgen - independent manner. This study focuses on BAG-1, a protein that is expressed in prostate cancers and which binds to androgen receptors, increasing their sensitivity to androgenic hormones. BAG-1 is a novel regulator of Hsp70 family molecular chaperones that was originally cloned in our laboratory. The activity of many steroid hormone receptors is known to be regulated by Hsp70 and Hsp90 family proteins. Recently, we have discovered that a previously unrecognized isoform of BAG-1, termed BAG-1L, can form complexes with androgen receptors (AR) and potentiate the actions of AR in prostate cancers, markedly lowering the concentrations of dihydrotestosterone needed for AR-mediated transactivation. In contrast, the shorter BAG-1 protein forms complexes with RAR family retinoid receptors, preventing retinoid-induced transactivation of target genes, abrogating cell growth suppression, and blocking apoptosis. Since BAG-1 and BAG-1L are commonly expressed in prostate cancers, we hypothesize that these proteins contribute to mechanisms of tumor resistance to anti-androgen and retinoid-based therapy. In this proposal, we describe experiments designed to further explore the biological roles and molecular mechanisms by which BAG-1 and BAG-1L regulate responses of prostate cancers to steroid hormones that control the proliferation, differentiation and survival of these malignancies.

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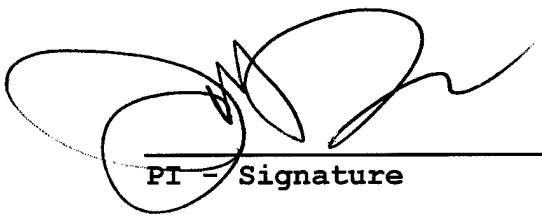

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John C. Reed, MD, Ph.D.

Regulation of Androgen Responses in Prostate Cancer by BAG-1

TABLE OF CONTENTS PAGE

INTRODUCTION

Prostate cancer is an androgen-driven disease. In the absence of testosterone or related androgens which can serve as ligands for the androgen receptor, the secretory epithelial cells of the prostate undergo rapid programmed cell death (1). Current treatment for metastatic adenocarcinoma of the prostate is predicated on the cell death inducing effects of anti-androgens and hormone ablative measures which reduce endogenous production of androgens. However, nearly all hormone-dependent prostate cancers eventually relapse as fatal hormone-independent disease (2).

Multiple, still largely unidentified mechanisms may account for the complete independence or reduced dependence of prostate cancers on androgens (reviewed in (3-5). AR gene deletion and sequestration of AR in the cytoplasm have been described in some hormone-independent tumors, implying that genetic alterations associated with tumor progression can abrogate the necessity for AR in some cases. However, many tumors may rely on other strategies which allow cancer cells to grow in low concentrations of androgens, including AR gene amplification or over-expression (6, 7) and AR mutations which permit trans-activation of target genes with little or no requirement for steroid hormones (4, 8). Since most hormone-insensitive prostate cancers still retain a wild-type AR, presumably alterations in the factors that control the levels of AR and its function play a major role in resistance to anti-androgen and hormone ablative therapies. Thus, a need exists to understand more about the molecular mechanisms that govern the activity of ARs.

Upon binding steroid ligands, the AR undergoes a conformational change, translocates to the nucleus and binds to specific DNA sequences located near or in promoter regions of target genes. After binding DNA, the receptor interacts with components of the basal transcription machinery and sometimes sequence-specific transcription factors, resulting in positive or negative effects on gene transcription (9, 10). A number of proteins have been identified which associate with hormone-receptor complexes, including several heat shock family proteins and various types of transcription co-activators (reviewed in (11, 12). However, many details remain unclear as to the molecular mechanisms by which these proteins modulate the activities of steroid hormone receptors and even less is known about whether alterations in their expression or function might contribute to the deregulation of steroid hormone responses in prostate cancer.

In this proposal, we describe experiments designed to explore the biological significance and molecular mechanisms by which AR is regulated by BAG-1 family proteins. BAG-1 is a novel Hsp70-family binding protein cloned in our laboratory. We have discovered that an isoform of this protein, BAG-1L, forms complexes with AR and potentiates the activity of this steroid hormone receptor, allowing it to transactive target genes with 100-1,000 x lower concentrations of dihydrotestosterone. We have also determined that BAG-1 and BAG-1L are expressed in most prostate cancers. Our goals now are to define the overall significance of BAG-1 family proteins on AR responses in prostate cancers and to delineate the mechanisms by which these Hsp70-binding proteins

control the function of AR and other steroid hormone receptors of relevance to prostate cancer cell growth, differentiation, and survival. These studies may reveal new strategies for improving androgen ablation therapy and attacking hormone-refractory metastatic prostate cancer.

BODY

OBJECTIVES

The original funded objectives of the project were to:

1. Determine the expression and location of BAG1 and BAG1L in primary and metastatic prostate cancer.

2. Study consequences of ablation of BAG1 and BAG1L expression in prostate cancer cell lines.

3. Examine in vivo effects of BAG1 and BAG1L on the androgen-dependence of the normal prostate gland.

PROGRESS

Objective #1. Determine the expression and location of BAG1 and BAG1L in primary and metastatic prostate cancer.

This aim has been partially accomplished. We generated monoclonal antibodies which recognize the BAG1 and the BAG1L proteins. We determined that BAG1 is cytosolic while BAG1L is nuclear. Using these monoclonal antibodies, we have use immunohistochemical methods and archival paraffin-embedded prostate cancer specimens to evaluate the expression of the nuclear (BAG1L) and cytosolic (BAG1) proteins in over 700 cases of prostate cancer. Comparisons were made with BAG1 immunostaining results in normal prostate and benign prostatic hypertrophy. Tissue microarray technology was exploited for much of this analysis, permitting us to analyze large numbers of tumor specimens.

BAG1 immunostaining was generally present in $\leq 30\%$ of normal prostate epithelial cells, with 51/54 normal specimens having $\leq 30\%$ BAG1 immunopositive epithelial cells. By comparison, most (91%) prostate cancers contained elevated percentages of BAG1 immunopositive cells, with 657 of 722 specimens (91%) having elevated percentages ($\geq 40\%$) of BAG-positive tumor cells. No correlations of BAG1 immunostaining data with Gleason grade or clinical stage. The percentages of BAG1 immunopositive cells were also not significantly different when comparing primary However, in men treated with radical prostatectomy (n = 160), log-rank analysis demonstrated an association between BAG1 immunostaining data and recurrence-free survival ($p = 0.009$), with higher percentages of BAG1 immunopositive cells associated with longer recurrence-free survival (median survival 4.6 versus 2.4 years). Further analysis of these data is underway, as we seek additional correlations of BAG1 immunostaining data with clinical endpoints.

From these observations, two conclusions can be reached. First, tumor-specific increases in BAG1 levels commonly occur in prostate cancers. Second,

compared to tumors that do not over-express BAG1, the expression of BAG1 may be associated with longer survival. However, since our antibodies do not discriminate between BAG1 and BAG1L, these results must be interpreted with caution. Efforts are underway to generate antibodies that react uniquely with the longer BAG1L protein, which is the isoform of the protein which has been shown to associated with AR and other nuclear hormone receptors.

Objective #2. Study consequences of ablation of BAG1 and BAG1L expression in prostate cancer cell lines.

To interfere with BAG1 and BAG1L function in prostate cancers, we are have expressed mutants of BAG1 or BAG1L which lack the C-terminal domain required for Hsc70-binding, so-called ΔC mutants. Our initial efforts have focused on assessing the impact of these trans-dominant inhibitory mutants of BAG1 and BAG1L on function of the Androgen Receptor (AR). These studies revealed that BAG1L(ΔC) suppresses the function of the AR, preventing it from transactivating reporter genes in transient transfection reporter gene assays.
An initial description of this work has been published by our group, but additional work in which we have extended the analysis to other cell lines has confirmed and solidified these impressions (13). An example of an experiment is presented in Figure 1, where various concentrations of a plasmid encoding BAG1L(ΔC) were compared with another plasmid producing a BAG1L mutant missing it N-terminal 50 aminoacids, BAG1L(ΔN). The BAG1L(ΔC) protein interfered with AR-induced activation of a reporter gene plasmids, whereas the BAG1L(ΔN) protein did not.

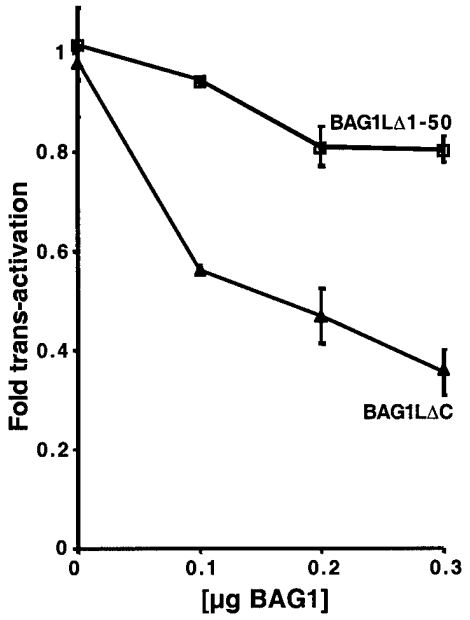

Figure 1. BAG1L(ΔC) functions as a trans-dominant inhibitor which interferes with AR function. COS-7 cells were transfected with 0.04μg of pSG5-AR, 0.5μg of pLCI, 0.06μg of pCMV-βGal, 0.03μg of pcDNA3-Bag1L and increasing amount of pcDNA3-Bag1LΔ1-50 or pcDNA3-Bag1LΔC. Total DNA was maintained at 1.4μg by the addition of empty plasmid. 30 hrs after transfection, cells were stimulated with 1nM R1881. Cell extracts were prepared and assayed for CAT and β-galactosidase activity at 40 hrs after transfection. Data are expressed as in Figure 1 (mean ± S.D., n=2). We are now attempting to generate stable transfectants in prostate cancer cell lines so that we may complete this Objective.

Objective #3. Examine in vivo effects of BAG1 and BAG1L on the androgen-dependence of the normal prostate gland.

Efforts to generate transgenic mice expressing BAG1 or BAG1L under the control of a probasin promoter are underway. Constructs have been prepared and are undergoing testing by transfection in prostate cancer cell lines in vitro before progressing to transgenic mouse production.

PUBLICATIONS

No papers resulted from the work performed during the past funding period, but a manuscript is in preparation which describes the immunohistochemical analysis of BAG1 expression in prostate cancers (Objective #1) and another paper will soon be prepared resulting from our studies of trans-dominant inhibitory mutants of BAG1L (Objective #2).

CONCLUSION

Understanding the molecular basis for progression of prostate cancers to hormone-refractory disease is critical for designing new therapeutic strategies for the treatment of advanced prostate cancer. We have discovered a protein, BAG1L, that binds the androgen receptor (AR) and enhances its resistance to anti-androgenic agents. Our findings accomplished with funding from this grant indicate that BAG1 expression is abnormally elevated in the vast majority of prostate cancers. Future studies of the impact of BAG1 overexpression and BAG1 inhibition in the prostate glands of transgenic mice will reveal the overall significance of BAG1 for regulation of androgen-responses in normal and malignant prostate tissue, and will help contribute to new strategies for overcoming hormone-resistant prostate cancer.

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